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MORPHOLOGY OF LEECH SENSILLA: OBSERVATIONS WITH THE SCANNING ELECTRON MICROSCOPE

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Abstract

The external morphology of the body wall of the medicinal leech, *Hirudo medicinalis*, was investigated with the scanning electron microscope. At high magnification, most of the leech body wall has a granular appearance punctuated by numerous small pores. The disc-shaped sensory sensilla are easily distinguished from the surrounding body wall by the absence of pores and the presence of numerous filiform projections in the central region of each sensillum. Two types of projections can be distinguished: single, 3–9 μm long "S-hairs" and grouped (*i.e.*, composed of several subunits), 1–2 μm long "G-hairs." Each sensillum supports 40–90 S-hairs and 15–20 G-hairs. The S-hairs may be the sensory structures mediating leech sensitivity to low amplitude water movements.

Introduction

Several types of sensory receptors occur in the leech body wall. One type, free nerve endings in the epidermal layer, has recently been shown to be the sensory terminals of touch-sensitive neurons (Blackshaw and Nicholls, 1979). Other cutaneous receptive endings must mediate sensitivity to pressure and to noxious stimuli (Nicholls and Baylor, 1968). In addition to these generalized tactile mechanoreceptors, leeches have localized receptors: eyes and segmental sensilla. The eyes, photosensory structures at the anterior end of the animal, may have sufficient complexity to provide some rudimentary vision of forms (Mann, 1961). In the medicinal leech, the segmental sensilla are 14 disc-shaped sensory structures on the middle one of five annuli that delineate each segment of the midbody region. Sensilla are also found in the head and tail regions.

It has long been thought that the segmental sensilla, as well, mediate light sensitivity (Whitman, 1886). This was demonstrated electrophysiologically by Kretz *et al.* (1976). As in leech eyes, the photoreceptors evidently consist of a few spherical refractile cells whose axons project centrally via the sensillar nerve (Whitman, 1886). In addition to these photoreceptors, the sensilla contain numerous elongated sensory cells, some of which appear to have filiform processes extending beyond the overlying cuticle (Mann, 1961). The function of these elongated sensory cells remains unknown, although Herter (1929) suggested that the sensilla may mediate sensitivity to water movements. Mann (1961) suggested that the sensilla also may mediate chemosensitivity.

Recent physiological experiments with medicinal leeches showed that the sensilla are indeed the locus for the sensory receptors mediating the sensitivity to water movements (Friesen, 1981). In this paper, we report the results of an investigation with the scanning electron microscope of the external morphology of segmental sensilla in the leech, *Hirudo medicinalis*. We found that midbody sensilla are

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composed of two morphologically distinct regions: (1) a central disc bearing two types of filiform processes, surrounded by (2) a pore-free, apparently raised ring not bearing any filiform processes. Leech sensitivity to water movements could be mediated by one of the two types of filiform processes.

Materials and Methods

Hirudo medicinalis specimens obtained from a commercial supplier were kept in aquaria at 20°C. The 20 leeches used for these studies were 7–10 cm long and had not been fed recently. Dissections were performed on leeches anesthetized by cold Ringer's solution (Friesen, 1981) containing 8% ethanol.

Two tissue preparations were used: whole leeches and body-wall sections composed of three or four segments. Mucus was removed from the tissue by brief rinsing in 8% ethanol. The tissue was then fixed with 2% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.5). During fixation, the sensilla were located by examining the body wall sections under the dissecting microscope. The tissue was then nicked at two edges so that the intersection of lines from the nicks would coincide with the position of the sensilla. This made it easier to find the sensilla when viewed with the scanning electron microscope (SEM) and made it possible to compare the structures seen with the light microscope with those observed with the SEM.

Following fixation, the tissues were rinsed in 3.5% sucrose in 0.1 *M* phosphate buffer. Following dehydration with ethanol, the whole leeches were cut into sections containing three or four body segments. After dehydration with a critical point dryer, the tissues were affixed to stubs with Pelca silver paste and coated with a 150–300-Å-thick layer of gold-paladium in a Technic Hummer I. The prepared tissue was examined with an ETEC Model II scanning electron microscope.

To assess shrinkage due to tissue preparation, the dimensions of annuli and sensilla were measured *in vivo*, with the leech in cold Ringer's solution, after fix-

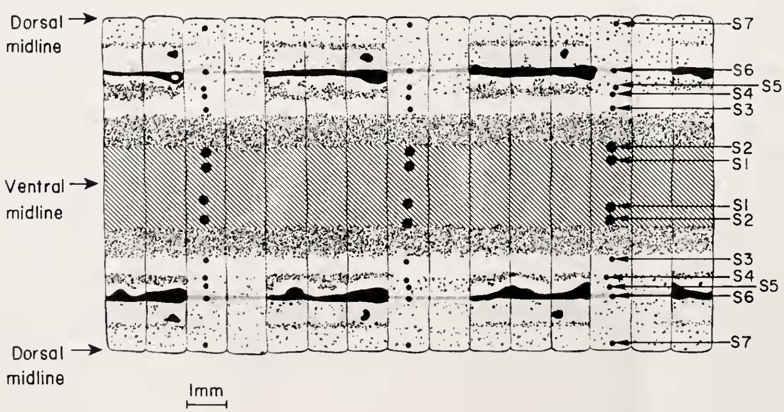
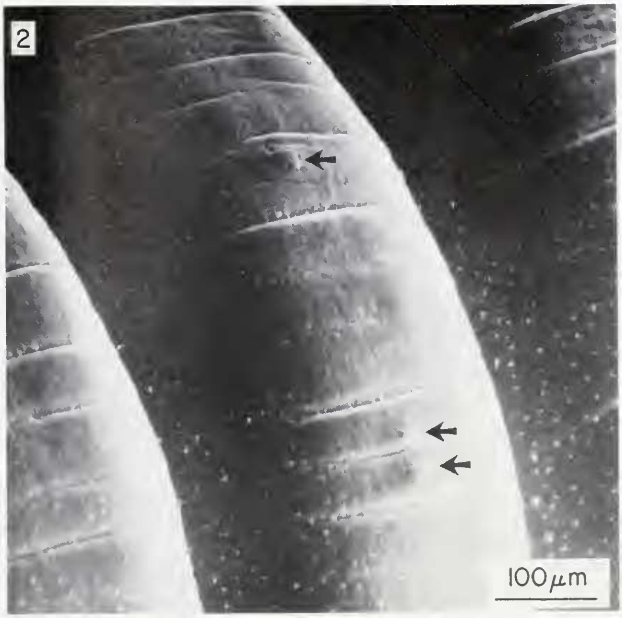


FIGURE 1. The medicinal leech body wall, drawn as viewed with the light microscope. The body wall was slit along the mid-dorsal line and three segments were pinned out flat, with the anterior to the left. Note that each body segment is subdivided into five annuli. The 14 sensilla are located on the middle annulus of each segment at the positions and with the relative sizes shown by the black dots. The stripes at the ventral midline represent yellow, small dots represent orange, and irregular black flecks and solid black represent black. The unmarked background color is green.



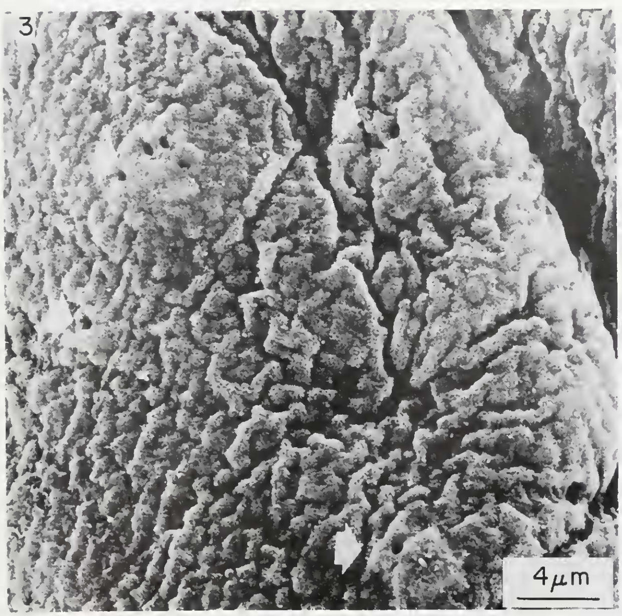


FIGURE 2. Low power SEM photomicrograph of the leech mid-body. The middle annulus of a midbody segment crosses the middle of the figure. The body wall is oriented so that the ventral midline is above the viewed area and anterior is to the left. The arrows indicate structures that occur at every fifth annulus.

FIGURE 3. High power SEM photomicrograph of typical body wall. Deep fissures and shallow grooves may result from muscular contractions. Between grooves, the body wall is characterized by shallow ripples and numerous pores (at arrows).

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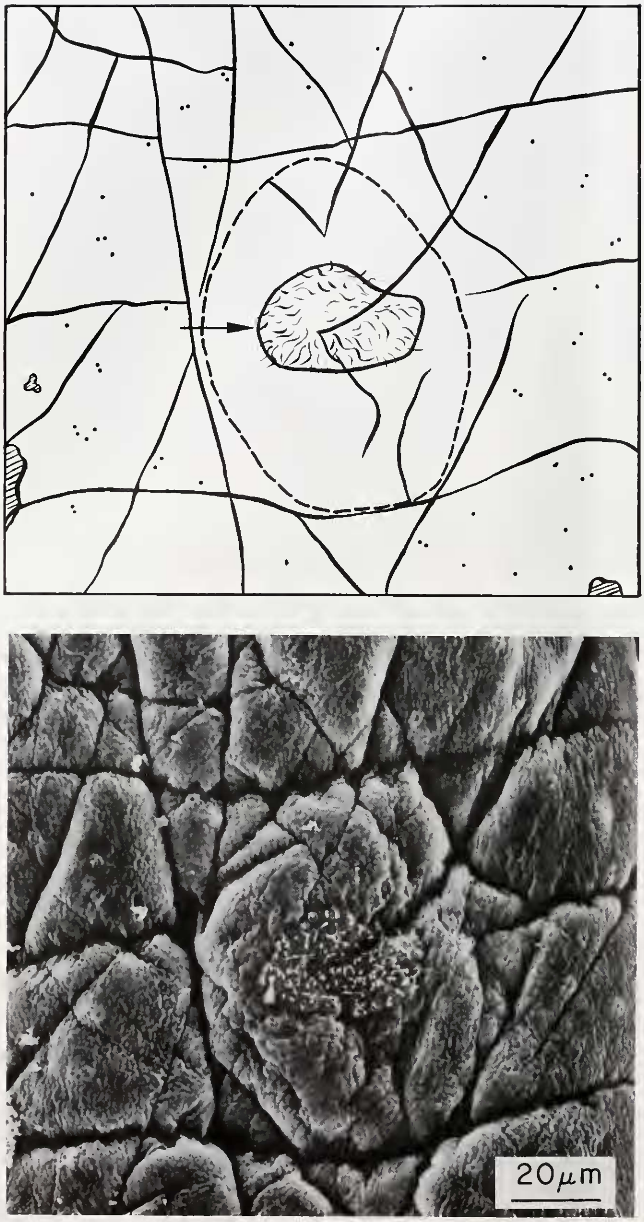

FIGURE 4. Appearance of the leech body wall at sensillum S1. The central area with numerous white projections is surrounded by a 20-μm-wide ring, which like the central area is devoid of pores. The sketch illustrates the extent of the two areas more clearly—the dashed lines indicate the approximate limit of the sensillum.

ation, while in the sucrose solution, and finally after critical point drying. All measurements were made with a dissecting microscope equipped with a reticule. One problem in evaluating dimensions in the leech is that the degree of contraction/

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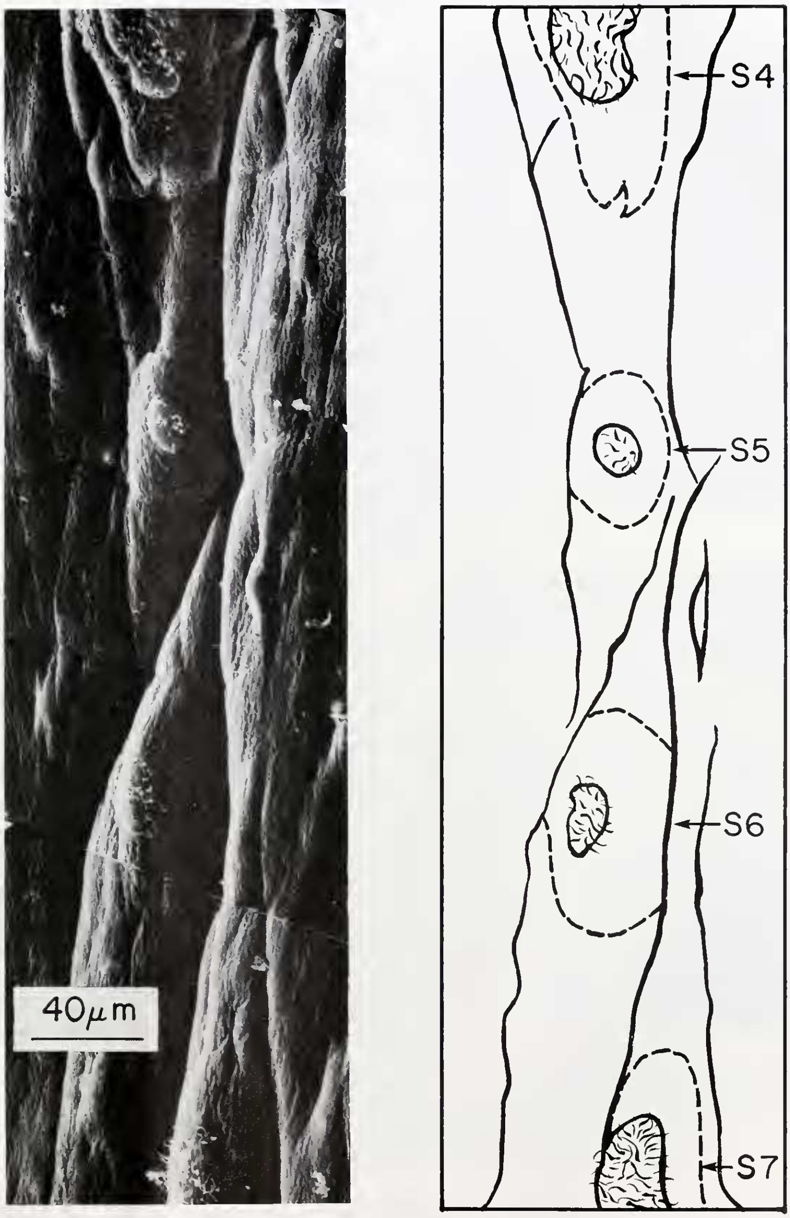


FIGURE 5. Photomicrograph of the middle annulus of the anterior midbody, showing four sensilla: S4, S5, S6, and S7. Both the central region and the surrounding raised ring of each sensillum are evident. The sketch at the right shows the extent of each structure.

relaxation varies not only from animal to animal but also along the individual leech itself. Waves of contraction still passed along the bodies of animals in cold Ringer's solution or in fixative, even though the animals were stretched out. To standardize

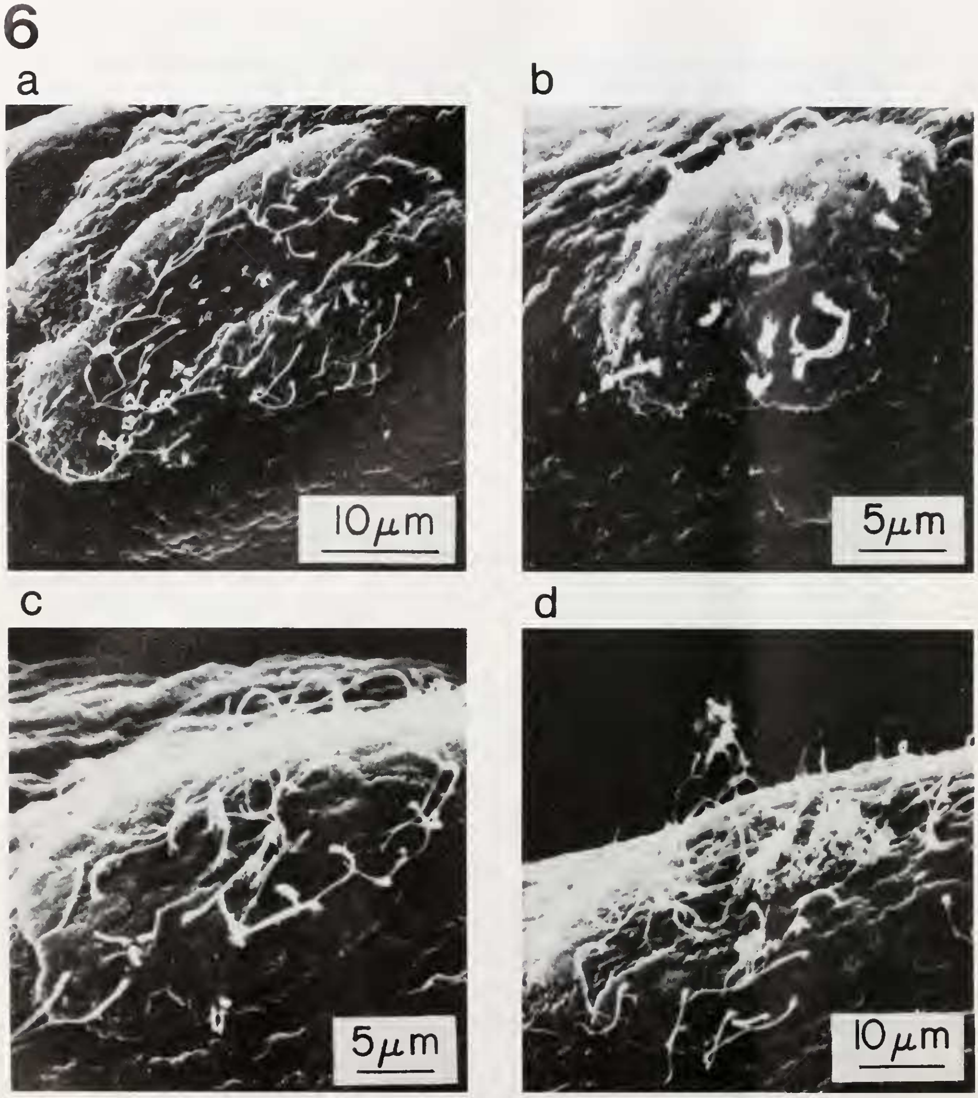
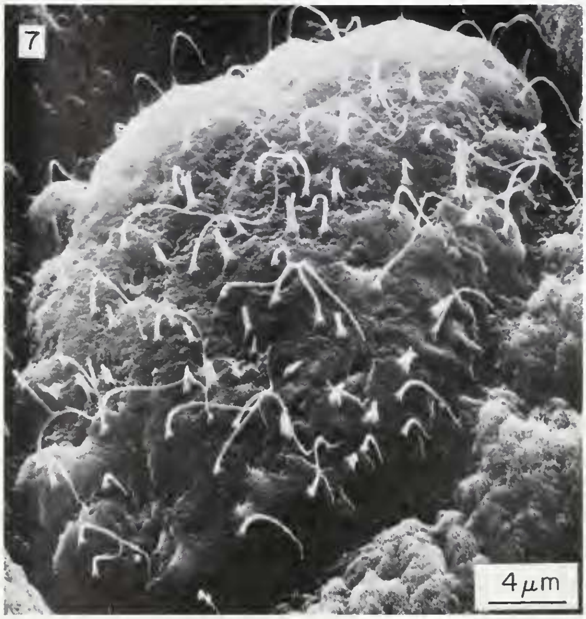


FIGURE 6. The sensilla of Figure 5 at greater magnification. The correspondence between these photomicrographs and the sensilla of Figure 5 is as follows: "a" shows S4; "b", S5; "c", S6; and "d", S7. Note that the viewing angles are not necessarily the same as that of Figure 5 and that magnifications differ.

the annular widths, we measured extended and contracted annuli, and averaged the difference between those two measurements for a number of annuli in the anterior, middle, and posterior regions. This average difference for a given region was then added to the measurements of contracted annuli. This procedure converted all annular widths to those of fully extended annuli. Comparing annular measurements for *in vivo*, fixed, and dried specimens showed that width decreased 16–20% during the drying process, comparable to the shrinkage observed by Hayat (1978).

The nomenclature used to describe leech segmentation is that of Kristan *et al.*



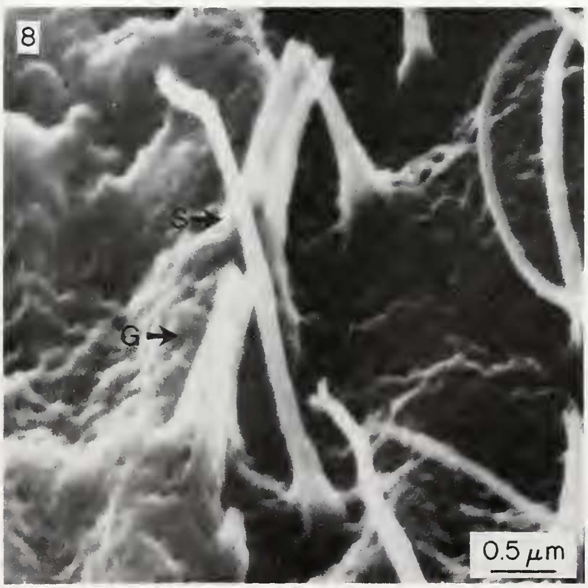


FIGURE 7. Photomicrograph of sensillum S3. Longer single hairs, S-hairs, and shorter grouped hairs, G-hairs, occur. The two hair types are interspersed evenly over the entire central region.

FIGURE 8. High power SEM photomicrograph of the S- and G-hairs located on sensillum S2. The G-hairs occur in tightly apposed groups of two or more and are 0.2–0.4 μm in diameter and 1–2 μm in length. The S-hairs measure 0.2–0.4 μm in diameter and 3–9 μm in length.

(1974). By this numbering scheme, segment 1 is the first of twenty-one midbody segments. We observed only these midbody segments (1 to 21). The sensilla are labeled S1 to S7, according to the scheme of Kretz *et al.* (1976). S1 and S2 are the ventral-most, S3 is positioned laterally, and S4–S7 are on the dorsal aspect of the leech body wall.

Results

Location and dimensions of sensilla

With the dissecting microscope, sensilla can be distinguished from the unspecialized body wall as light grey or semitransparent circular to elliptical areas on the middle annulus of each midbody segment. The actual shape of the sensilla varies depending upon the degree of contraction of the surrounding body wall. In Figure 1, the ventral-most sensilla (S1 and S2) are easily discernable in the light yellow ventral body wall. S1 lies approximately 500 μm from the ventral midline, and S2 about 400 μm more laterally. S3 is in the light yellow band of the lateral edge, sometimes almost within the dark ventrolateral band. S4 and S5 lie in the black and green coloring just dorsal to the lateral yellow band. These two sensilla may be as little as 100 μm apart. The next sensillum, S6, is in the dorsolateral orange band, about one-third of the way from S5 to S7. Finally, S7 is located about 200 μm from the dorsal midline, within the dorsal band. Because of bilateral symmetry, each sensillum has a homonymous counterpart located in the contralateral body wall.

The sensilla fall into two size classes: about 100 μm $\times$ 200 μm for the diameters of the minor and major axis of S1 and S2 and about 60 μm $\times$ 90 μm for the same dimensions of S3 to S7. Because S1 and S2 are larger and situated in uniformly colored body wall, they are the sensilla most easily discernible with the dissecting microscope.

The leech body wall is very distendable. Sensillar dimensions are therefore only approximate; their deviation from roundness and their apparent size are altered by changes in stretch or contraction of the body wall.

When viewed with the SEM under low magnification, the external body wall appears as a series of rounded ridges—the annuli seen with the unaided eye and the light microscope. Figure 2 shows the lateral aspect of the leech body wall. The rounded band running across the middle of this photomicrograph is the middle annulus of a midbody segment. Parts of adjacent annuli can be seen to either side of this annulus. Numerous crevices in the annuli parallel the anterior-posterior axis of the leech. The small rounded structures (at arrows) mark the location of the sensilla. These structures were observed at the middle annuli of all segments, but not on the intervening annuli.

Identification of the sensilla with the SEM

Examination of the leech body wall with the SEM at high magnification reveals numerous small grooves and fissures (Fig. 3). Between these, the surface is fairly smooth, though still somewhat rippled and generally grainy, possibly due to epidermal projections such as have been described in other leech species (Desser and Weller, 1977). Small pores (arrows, Fig. 3) found over most of the body surface, usually in groups of two or three, probably are the openings of the unicellular epidermal glands observed with the light microscope (Mann, 1961).

Figure 4 is a medium power SEM photomicrograph of a preparation marked to identify its sensilla as those seen with the dissecting microscope. The body wall surface near the perimeter is divided by fissures, appears rippled, and has numerous pores at irregular intervals. However, the center of the picture—at the marked location—is quite different. First, an oval area with white, elongated projections (at arrow in the line drawing) can be seen at the very center. Second, this central area and a surrounding ring are devoid of pores. These two features distinguish the sensilla from the general body wall. This body wall morphology was found in all the marked body wall sections examined. It also was found when the small round structures shown in Figure 2 were examined at high magnification, demonstrating that these structures are, in fact, sensilla.

The composite photomicrograph of Figure 5 shows four sensilla from the middle annulus of one midbody segment at medium magnification. The accompanying sketch illustrates the limits of the sensillar areas. While the body wall pores are not evident at this magnification, the central area of each sensillum is surrounded by a ring of tissue that evidently is slightly elevated from the surrounding body wall.

Sensillar substructure

The question of whether the two morphologically distinct regions at the sensilla both make up the sensillar disc seen with the light microscope was answered by comparing the dimensions of the structures. The average diameter of the central region of the structure seen with the SEM is about 20 μm, much smaller than the sensillar diameters measured with the light microscope, even allowing for shrinkage. Thus, the central sensillar region bearing the white projections is a distinct substructure of the sensilla. The raised, pore-free ring surrounding this central region varies in width from about 10 to 40 μm. The total diameter of the sensillar structures observed with the SEM is thus about 40 to 100 μm—not much less, when allowance is made for tissue shrinkage, than the sensilla diameters measured with the dissecting microscope. Thus, the central region and its surrounding ring observed with the SEM probably correspond directly to the grey discs seen with the light microscope.

Figure 6 shows at higher magnification the sensilla in Figure 5. The central region is obvious as a raised area and the white projections appear as filiform processes from the central region.

Filiform projections

Filiform projections from leech sensilla are known from previous studies with the light microscope. Illustrations from Mann (1961) indicate that these projections are found only at the central area of a sensillum, as our SEM micrographs also indicate. As Figure 7 shows, there are two distinct types of filiform processes: some are long and occur singly; others are shorter and occur as groups of two or more subunits. We have labeled the single projections "S-hairs" and the grouped structures "G-hairs." Both types appear to be evenly distributed over the central region without segregation by type. The S-hairs appear bent, suggesting flexibility, while the G-hairs have a more rigid, rod-like appearance.

The hairs' detailed structure is more evident at the high magnification of Figure 8. The S-hairs taper from a diameter of about 0.4 μm where they leave the body wall to a diameter of about 0.2 μm at their tips. The S-hairs at the ventral sensilla (at S1 and S2) are relatively short (1–3 μm long). At the more dorsal sensilla (S3–S7), these hairs are longer (4–9 μm). Because of foreshortening from the projection of a three-dimensional object onto a two dimensional surface, these measured lengths are minimum values. The G-hairs derive their appearance from the close apposition of two or more projections that individually resemble short S-hairs. The length of G-hairs at all sensilla is nearly equal (1–2 μm), considerably shorter than the dorsal S-hairs but only slightly shorter than the ventral S-hairs.

The hairs are not distributed uniformly. The ventral-most sensillum, S1, has 89 (SD = 10, N = 8) S-hairs and 18 (SD = 5, N = 8) G-hairs, S2 has 60 (SD = 9, N = 9) S-hairs and 17 (SD = 4, N = 9) G-hairs, S3 has 54 (SD = 7, N = 7) S-hairs and 20 (SD = 7, N = 7) G-hairs, and S4–S7 (dorsal sensilla) have 40 (SD = 22, N = 14) S-hairs and 16 (SD = 9, N = 14) G-hairs. Thus, while the number of G-hairs per sensillum is nearly constant, there are more S-hairs per sensillum on the ventral body wall.

Discussion

Two new aspects of the external morphology of leech sensilla are reported here. First, the SEM photomicrographs show that the sensilla have two distinct substructures: a central raised region with a diameter of about 20 μm, which bears numerous filiform projections; and a raised ring about 10–40 μm wide surrounding this central region. In contrast to the general body wall, the sensilla are devoid of pores. Second, the filiform processes observed at the sensillum fall into two distinct classes: single, relatively long hairs (S-hairs) and shorter hairs found in groups of two or more (G-hairs).

The central regions of leech sensilla resemble in size and general appearance the earthworm "sensory buds" described by Moment and Johnson (1979). In both of these annelids, these sensory structures are round, raised areas bearing numerous projections. And in both, these structures encircle the midbody regions on regularly spaced annuli. The sensilla of the medicinal leech, however, have two types of projections, while only one type of projection, single hairs, has been observed at the sensory buds of the earthworm (*Eisenia foedita*) (Moment and Johnson, 1979). The earthworm's single hairs resemble more closely the individual component projections of the short leech G-hairs than the longer, single S-hairs.

Recent physiological experiments (Young, Dedwylder, and Friesen, in press) have confirmed earlier studies by Herter (1929) that leeches are responsive to water vibrations. In particular, it was found that quiescent, submerged leeches will initiate locomotory activity in response to the stimulation provided by surface water waves. In the leech midbody, sensitivity to such wave stimulation is confined to the sensilla (Friesen, 1981). The filiform projections on the leech body wall are the best candidates for the receptors mediating vibration sensitivity, both because of their location at the sensilla and because of their similarity to other filiform processes known or suspected to be vibration receptors in vertebrates (Flock, 1965) and invertebrates (Tautz, 1979). Of the two hair types, the longer S-hairs appear better suited for transmitting the stimulus provided by the movements of water particles to sensory neurons.

Because of boundary layer effects, the amplitude of vibratory movements of water particles within 10 μm of the leech body wall is very small. For a filiform sensory structure to transmit vibrational energy effectively from the medium to the neuronal transducer, it must project beyond the stationary boundary layer (Tautz, 1979). Thus, the leech S-hairs, which appear no longer than 10 μm, must extend beyond the boundary layer to serve as sensory structures. This problem appears to have been solved in the leech by placement of the hairs at the sensilla, papillar structures that can be protruded from the body surface (Mann, 1961). In the medicinal leech, the sensilla can protrude 50 μm or more from the body wall (personal observations). Thus, the S-hairs appear to be suited for the role of water movement receptors.

Acknowledgments

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LITERATURE CITED

Blackshaw, S., and J. Nicholls. 1979. Distribution and morphology of touch cell endings in leech skin. *J. Physiol.* **292:** 26P–27P.

Desser, S. S., and I. Weller. 1977. Ultrastructural observations on the body wall of the leech, *Batracobedella picta. Tiss. Cell* **9:** 35–42.

Flock, A. 1965. Electron microscopic and electrophysiological studies on the lateral line canal organ. *Acta Oto-Laryn. Suppl.* **199:** 1–90.

Friesen, W. O. 1981. Physiology of water motion detection in the medicinal leech. *J. Exp. Biol.* **63:** In press.

Hayat, M. A. 1978. Pp. 134–136 In *Introduction to biological scanning electron microscopy*. University Park Press, Baltimore.

Herter, K. 1929. Reizphysiologisches Verhalten und Parasitismus des Entenegels *Protoclepsis tesselata* O. F. Mull. *Z. Vergl. Physiol.* **10:** 272–308.

Kretz, J., G. S. Stent, and W. B. Kristan. 1976. Photosensory input pathways in the medicinal leech. *J. Comp. Physiol.* **106:** 1–37.

Kristan, W. B., G. S. Stent, and C. A. Ort. 1974. Neuronal control of swimming in the medicinal leech. I. Dynamics of the swimming rhythm. *J. Comp. Physiol.* **94:** 97–119.

Mann, K. H. 1961. *Leeches*. Pergamon Press, London. 201 pp.

Moment, G. B., and E. Johnson, Jr. 1979. The structure and distribution of external sense organs in newly hatched and mature earthworms. *J. Morphol.* **159:** 1–16.

Nicholls, J. G., and D. A. Baylor. 1968. Specific modalities and receptive fields of sensory neurons in the CNS of the leech. *J. Neurophysiol.* **31:** 740–756.

Tautz, J. 1979. Reception of particle oscillation in a medium—an unorthodox sensory capacity. *Naturwissenshaften* **66:** 452–461.

Whitman, C. O. 1886. The leeches of Japan. *J. Micr. Sci. N.S.* **26:** 317–416.